Regional Transport & Pharmacology

Intro

In the last lesson we filtered plasma. Water, ions, glucose, and some proteins are pushed into the glomerulus. These substances would end up in the urine if the tubules did not act. Water and ions must be reabsorbed. The different segments of the nephron are designed to reabsorb specific substances. Specifically, some segments are impermeable to water but very permeable to ions, while other segments are permeable to water but not ions. Hear this loud and clear—water regulation and ion regulation are handled by different, nonoverlapping segments. This lesson ignores the segments that handle water (we'll take care of that in Kidney #5: *Water*), and focuses on the segments that reabsorb ions. In reality, of course, water and ions are intensely interconnected. Forcibly separating them into two lessons makes comprehending the pieces of that interconnectivity easier. Stay focused on the physiology and the pharmacology of ion transport.

If ions are moving out of the lumen of the tubules in these segments, and the tubules in these segments are impermeable to water, the concentration of the urine must go down. Said simply, if stuff is removed from water, there is less stuff per unit of water, and therefore the concentration is less. The segments that move ions but not water are the proximal convoluted tubule (PCT), the thick ascending loop of Henle (TAL), and the distal convoluted tubule (DCT). These are therefore said to be the diluting segments. The collecting duct (CD) moves ions and also water.

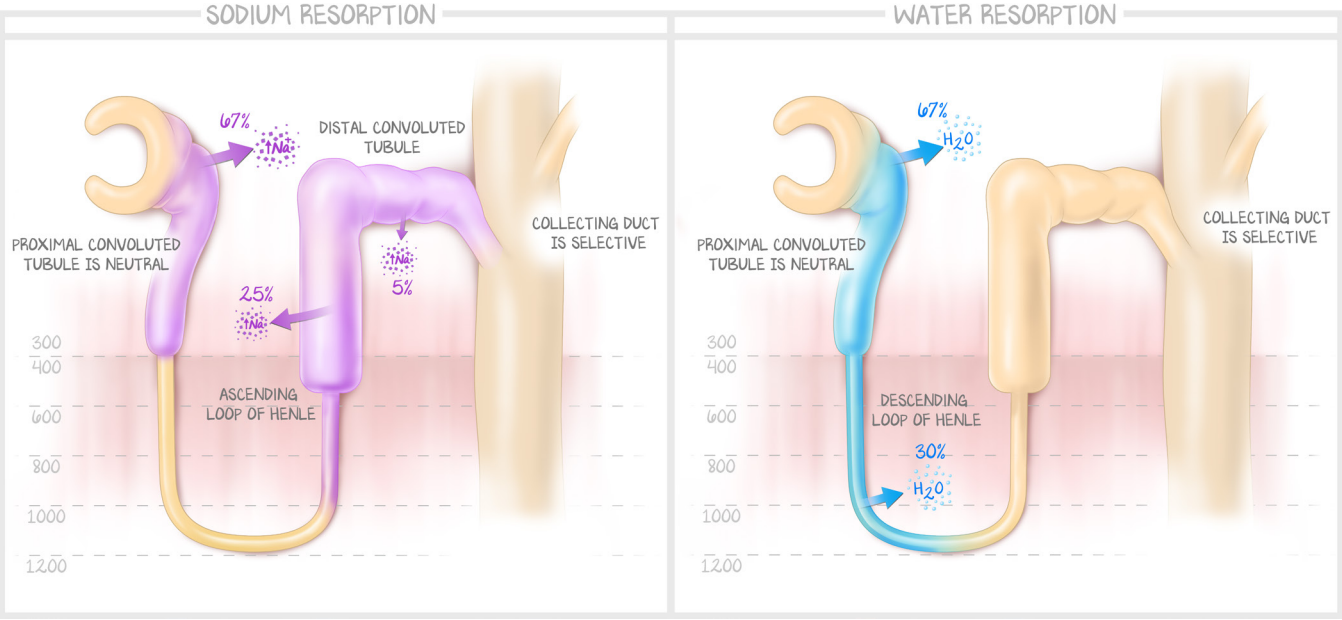
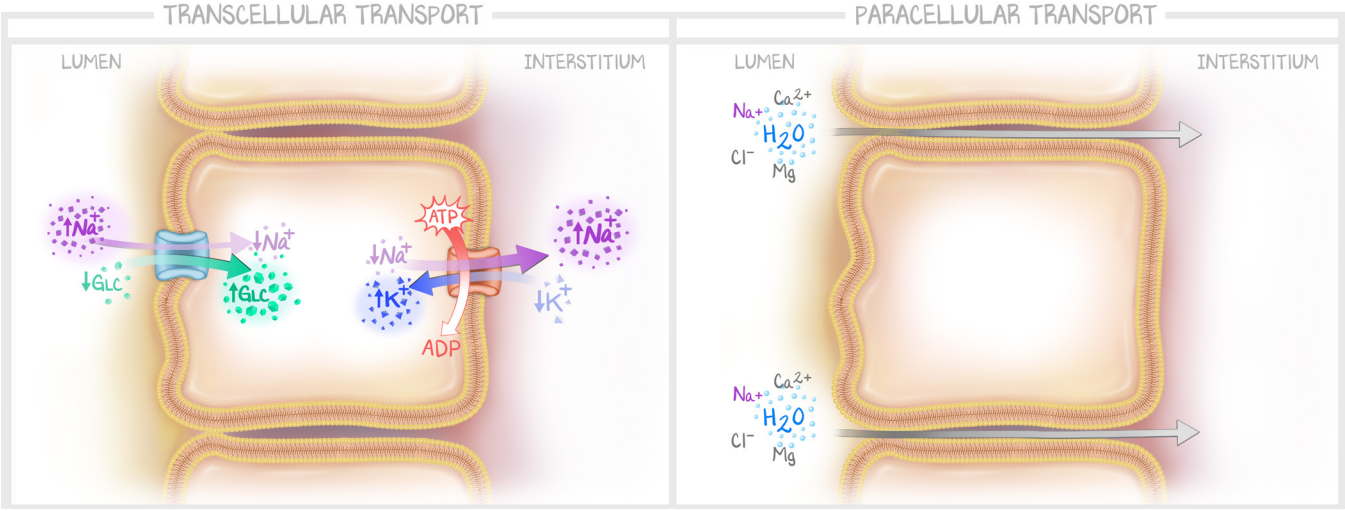


Figure 4.1: Segments of Ion and Water Reabsorption
A massive amount of water and ions is filtered by the glomerulus. Almost all of that needs to be reabsorbed by the tubules. For most filtered molecules that will be reabsorbed, 100% is reabsorbed by the proximal convoluted tubule. For water and sodium, only about two thirds, 67%, is absorbed by the PCT. The remaining water is absorbed by the descending loop of Henle (30%) and the collecting duct (< 5%). The remaining sodium is reabsorbed by the thick ascending limb (25%), the distal convoluted tubule (5%), and the collecting duct (< 5%). The collecting duct is the only segment with regulation.

The movement of all molecules from the lumen into the cells is driven by the sodium concentration gradient. There is a lot of sodium in the lumen. Sodium drives secondary active transport of other molecules in most segments that reabsorb ions. In order to establish a favorable concentration gradient, the **Na^+/K^+-ATPase** must work overtime, around the clock, at the basolateral membrane. Such intense metabolic work requires a large supply of **oxygen**. Every segment has **Na^+/K^+**-ATPase on its basolateral membrane.

Ions can move from the lumen into the interstitial fluid, then into the bloodstream, by two mechanisms of transport: paracellular and transcellular.

Paracellular means that ions will have to go between (para-) the cells (-cellular). Paracellular reabsorption ignores the epithelium of the tubules altogether. The epithelium is tightly connected (zona occludens, zona adherens), but the seal between cells is not perfect. Very small molecules—individual ions and water—can squeeze between epithelial cells. These substrates, having no channels or transport mechanisms, must inherently passively diffuse down their electrochemical gradients. Paracellular movement of ions helps explain some of the side effects of drugs, such as loop diuretics. The channel that loop diuretics block is on the apical surface of the tubule epithelium. The channel normally reabsorbs sodium, potassium, and chloride. Blocking that channel disrupts not only that channel, but subsequent paracellular reabsorption of magnesium. More on this later.



(a) (b)

Figure 4.2: Cellular Transport
(a) Transcellular transport harnesses the sodium concentration gradient established by a **Na^+/K^+**-ATPase on the basolateral side. This transport is regulated by apical transporters, and is selective. (b) Paracellular transport is regulated by passive diffusion between the lateral domains of the epithelial cells.

Transcellular absorption means that ions will have to go through (trans-) the cells (-cellular). The epithelium has polarity—the apical domain is in contact with the tubule lumen, and the basal domain in contact with the basement membrane, the kidney interstitium, and blood vessels nearby. On the apical domain, channels are designed for reabsorption. Almost all the transport into the cytoplasm from the lumen is via **secondary active transport**. No ATP is needed for these apical channels. But active transport moves substrates up their concentration gradient, and so requires energy. The energy to do that comes from **sodium gradients**. The movement of sodium into the cell generates the energy to move other molecules, such as glucose and ions. But that means the sodium gradient must constantly be replenished, maintaining a favorable gradient into the cell. That sodium concentration gradient is established by the basolateral **Na^+/K^+-ATPase**. And because excess water and solutes are filtered (to

ensure filtration and elimination of toxins), excess water and solutes need to be selectively reabsorbed. That means the tubule epithelial cells are intensely metabolically active and **require significant oxygen**.

Most reabsorption happens transcellularly. Only small ions and water (where the tubule is permeable) can sneak by paracellularly.

Proximal Convoluted Tubule

This is the most difficult, most potent, and most commonly tested segment of the tubules. Because it is the first segment of the nephron, it receives a higher quantity of substrate than anywhere else in the tubule. While some substances are filtered, reabsorbed, and excreted, most substrates get filtered then selectively reabsorbed. Reabsorption should happen only to substrates filtered out in order to filter toxins. The PCT, therefore, is the **site where the most reabsorption happens** for everything that gets filtered.

The PCT reabsorbs two-thirds of the filtered sodium, two-thirds of the filtered calcium, two-thirds of filtered potassium, two-thirds of the filtered water, 80% of filtered phosphate, and ALL of the filtered glucose and amino acids. Get a feel for the magnitude of that reabsorption. You probably already know that there are dilution segments and concentration segments, and that the collecting duct is the decider of urine volume and urine concentration. While the changes the collecting duct makes do have a perceivable influence on how the human body works (lots of diluted urine or very little concentrated urine), the amount of sodium and water the collecting duct handles is a mere fraction of what the PCT handles. We're going to cover some high-yield specifics of the PCT.

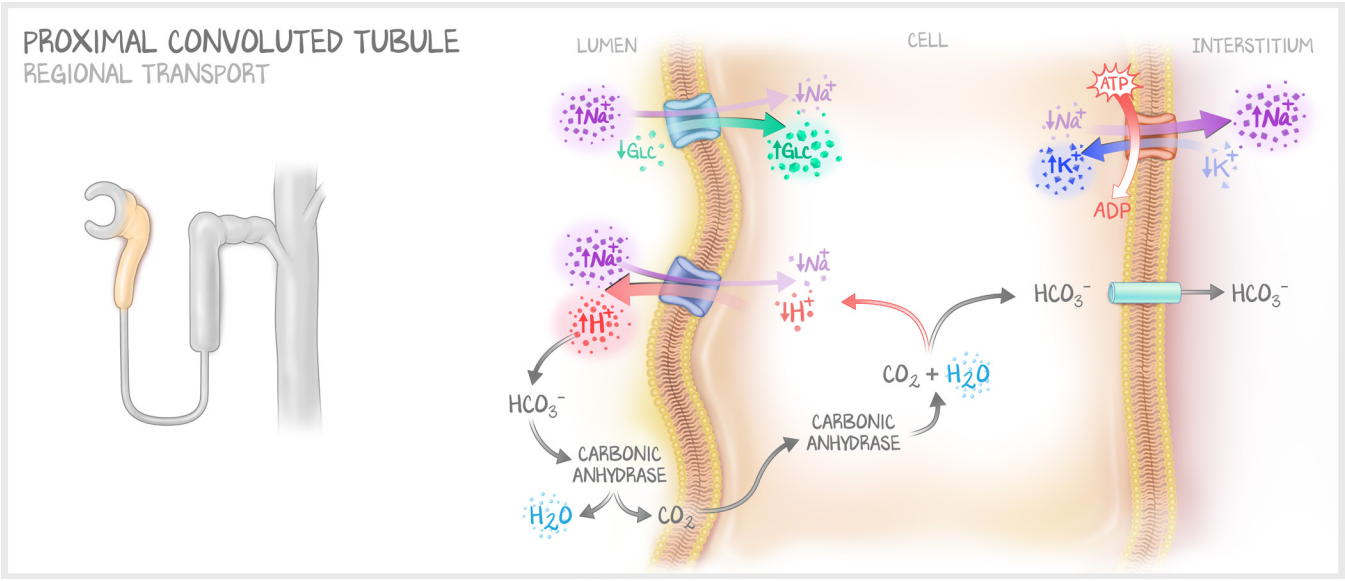


Figure 4.3: PCT Regional Transport
The PCT reabsorbs most of the molecules the nephron reabsorbs. It is the most permeable, has the most complicated channels, and receives the largest load of solute and water. In particular, the SGLT-2 transporters harness sodium concentration gradients into the cell to bring with it glucose. In addition, H^+ ions are used to drive Na^+ into the cell. Carbonic anhydrase in the lumen regenerates the CO_2 that re-enters the epithelial cell, and encounters another carbonic anhydrase, which regenerates the H^+. In this way, secondary active transport, antiport, continues to reabsorb sodium.

Glucose. The **SGLT-2** (sodium-glucose linked transporter) harnesses sodium's concentration gradient to bring glucose into the cell against its concentration gradient. The T_{max} of SGLT-2 is about 400 mg/min, which corresponds to about a 400 mg/dL blood sugar level. Around 200 mg/dL, glucose begins to

spill into the urine. Even though the PCT transporters have not reached their T_{max}, they are unable to reabsorb all the glucose. This is called splay. After 400 mg/dL, there cannot be any more absorption, and as glucose levels continue to rise in the blood, glucose levels continue to rise in the urine. Because normal blood glucose levels are around 100 mg/dL, the PCT in healthy patients always reabsorbs all the urine. Only in diabetes (discussed in detail in the Endocrine module) does sugar end up in the final urine.

Sodium. Sodium is used as the driver for absorption of all other substrates. As sodium is absorbed, the osmolarity of the tubule fluid should rise. However, the PCT always delivers an **isotonic tubule fluid** to the descending loop of Henle. It does that because water follows salt, so water and sodium are reabsorbed in equal amounts. Thus, the concentration in the tubule never changes.

Carbonic anhydrase. One way sodium is reabsorbed in the PCT is via a **Na^+-H^+ antiporter**, in which a sodium is brought in from the lumen and a hydrogen ion is released into the lumen. In the lumen, the hydrogen ion (H^+) combines with bicarbonate (HCO_3^-) to form carbonic acid, H_2CO_3. Luminal **carbonic anhydrase** (an-hydro-ase) removes H_2O from carbonic acid, leaving behind CO_2. The CO_2 diffuses passively back into the PCT epithelial cell, where it is rehydrated by cytoplasmic **carbonic anhydrase** to H_2CO_3. This quickly dissociates into a hydrogen ion (which is used for another cycle of the antiporter for sodium) and bicarbonate ion (which is pumped out into the interstitium and into the vasa recta). The bicarbonate that was filtered and paired with the original hydrogen ion in the lumen was just effectively reabsorbed.

Descending Limb and Thin Ascending Limb

Most of the loop of Henle is permeable to water and impermeable to ions. It is used to establish the concentration gradient that allows for water regulation, for concentrating or diluting the urine in the collecting duct. This is the countercurrent exchange discussed in Kidney #5: *Water*.

Thin Ascending Limb

The thin ascending limb is impermeable to water but permeable to sodium. As the limb ascends, the filtrate within the tubule is hypertonic to the surrounding interstitium. Because no water can move, sodium moves instead. Sodium exits the tubule passively, thereby diluting the filtrate in this segment. There are no pathologic states or pharmaceuticals that target this segment, so we have been ignoring it.

Thick Ascending Limb

In the thick ascending limb, the apical "sodium-potassium-dichloride" (**Na-K-2Cl) cotransporter** harnesses the concentration gradient of sodium to generate the energy to absorb the other ions. Potassium and chloride move up their concentration gradients. Every one unit of reabsorption by this transporter is electrically neutral (Na is 1+, K is 1+, and 2Cl is 2–). In addition to the basolateral K leak channel (found in every membrane where there is a **Na^+/K^+**-ATPase), there are also **apical K leak** channels. Potassium has a concentration gradient that favors K's leaving the cell. The electroneutral reabsorption of the Na-K-2Cl transporter leaves the lumen without a change in charge. With the passive leak of potassium back into the lumen, the lumen **gains positive charge**. This generates an electrical gradient that favors positive ions leaving the lumen. Specifically, it **favors paracellular reabsorption** of Ca^{2+} and Mg^{2+}, getting positive charges out of the lumen and into the plasma.

The TAL is **impermeable to water**. So, as ions are removed, the lumen becomes diluted. The thick ascending limb is therefore responsible for reabsorption of Na, Cl, Ca, and Mg. Blocking the Na-K-2Cl channel, therefore, results in a variety of electrolyte imbalances (later this lesson).

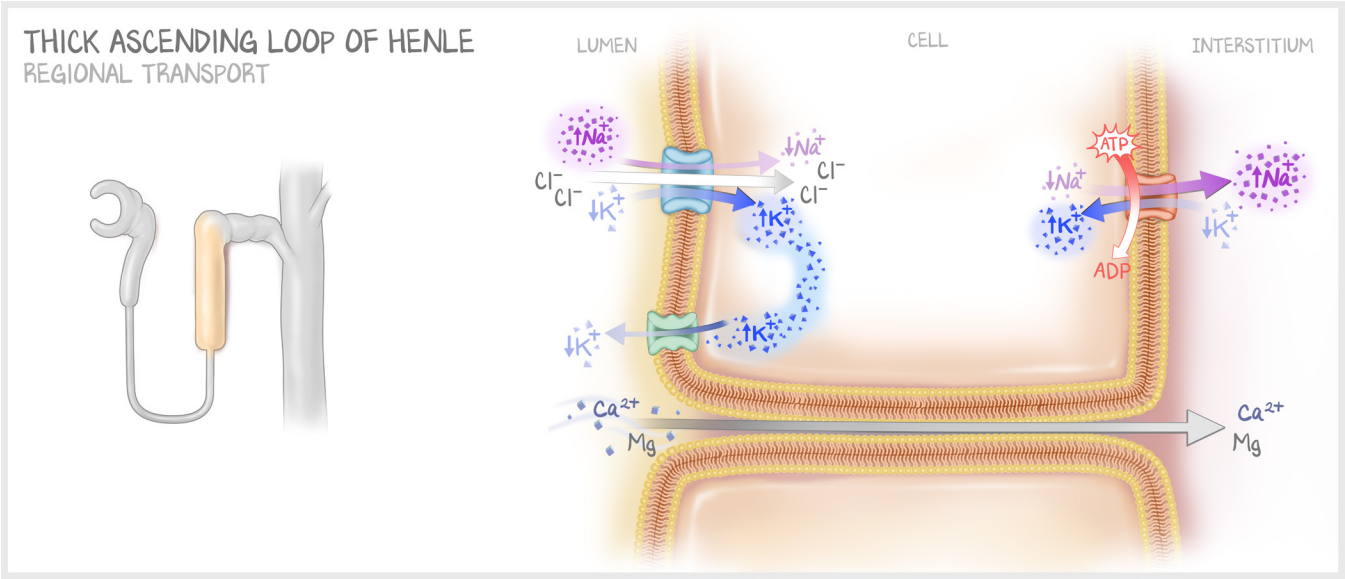


Figure 4.4: TAL Regional Transport
The Na-K-2Cl cotransporter harnesses sodium to bring in potassium and chloride. Potassium leaks out into the lumen, inducing paracellular reabsorption of Ca^{2+} and Mg^{2+}.

Distal Convoluted Tubule

The DCT has an apical **Na-Cl** cotransporter which harnesses sodium's movement down its concentration gradient to move chloride up its concentration gradient. Electrically neutral, Na^+ and Cl^- enter. There are no leak channels back into the lumen, so no real concern for paracellular shifts. Sodium is pumped out by the **Na^+/K^+**-ATPase. Chloride comes along for the ride (aka don't worry about chloride). The DCT is **impermeable to water** and is also a diluting segment.

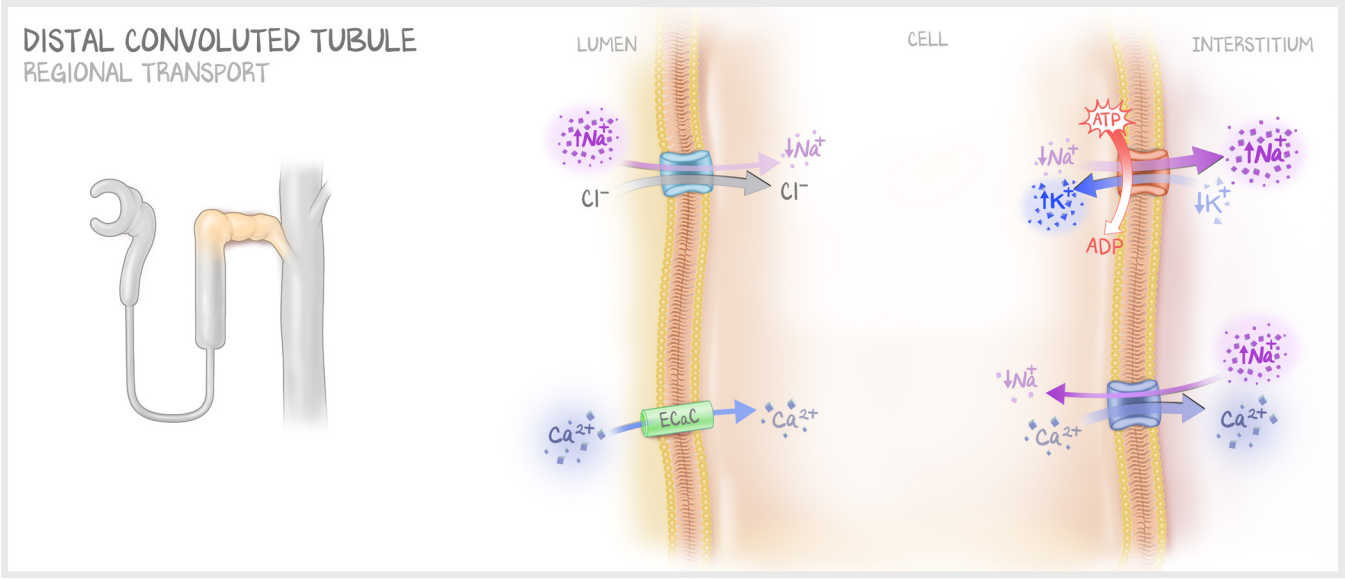


Figure 4.5: DCT Regional Transport
Two mechanisms are in play at the DCT—chloride and calcium. Na-Cl cotransport facilitates chloride absorption. The more sodium reabsorbed, the more chloride reabsorbed. Separately, calcium moves down its concentration gradient through ECaC channels on the apical membrane. The sodium gradient is used on the basolateral membrane to pump calcium out of the cell against its concentration gradient.

But what makes the DCT special is that it **also** is involved in **active calcium reabsorption**. Unlike the thick ascending limb, which uses paracellular shifts to absorb calcium, the DCT reabsorbs calcium transcellularly. Under the influence of parathyroid hormone (in the Endocrine module, *Parathyroid Hormone Physiology*), apical calcium channels open. Calcium moves down its concentration gradient from the lumen into the cytoplasm. But here is the tricky part. Getting calcium into the blood requires going up its concentration gradient, which requires energy. Calcium export at the basolateral membrane is via a **sodium-calcium antiporter**. Three sodiums enter the cell from the interstitium down their concentration gradient, and one calcium is pumped out, against its concentration gradient. This is a very similar mechanism to what we've been discussing, except that this antiporter is **basolateral**, not apical.

The DCT is therefore responsible for reabsorption of calcium, sodium, and chloride.

Collecting Duct

The collecting duct does a number of things. First, it passively reabsorbs sodium through ENaC ("EE-nack") when instructed to do so by aldosterone. Second, it is responsible for secreting hydrogen ions, again when instructed to do so by aldosterone. Third, unrelated to ions, the collecting duct can become permeable to water by the insertion of aquaporin channels under the influence of ADH. There are two types of cells in the collecting duct. **Principal cells** absorb sodium and water. **Intercalated cells** secrete hydrogen ions.

ENaC channels are transmembrane proteins. **Aldosterone** is secreted from the adrenal cortex in response to angiotensin-2 stimulation. Aldosterone is a steroid hormone and binds to **cytoplasmic receptors** which translocate and increase expression of ENaC channel genes. There is a favorable concentration gradient for sodium to enter the cell, established by the basolateral **Na^+/K^+**-ATPase. ENaC channels reabsorb only sodium via passive diffusion down sodium's concentration gradient. There is no harnessing this energy, as there is in other segments. But the entry of a positively charged ion creates an electric gradient that favors positively charged ions to leave the cell. There is an **apical K leak channel**, that, when there is an increased electrical gradient, balances the cell's charge by **potassium leaving the cell** into the lumen. The principal cells can therefore be seen as **reabsorbing Na and secreting K** in response to aldosterone stimulation.

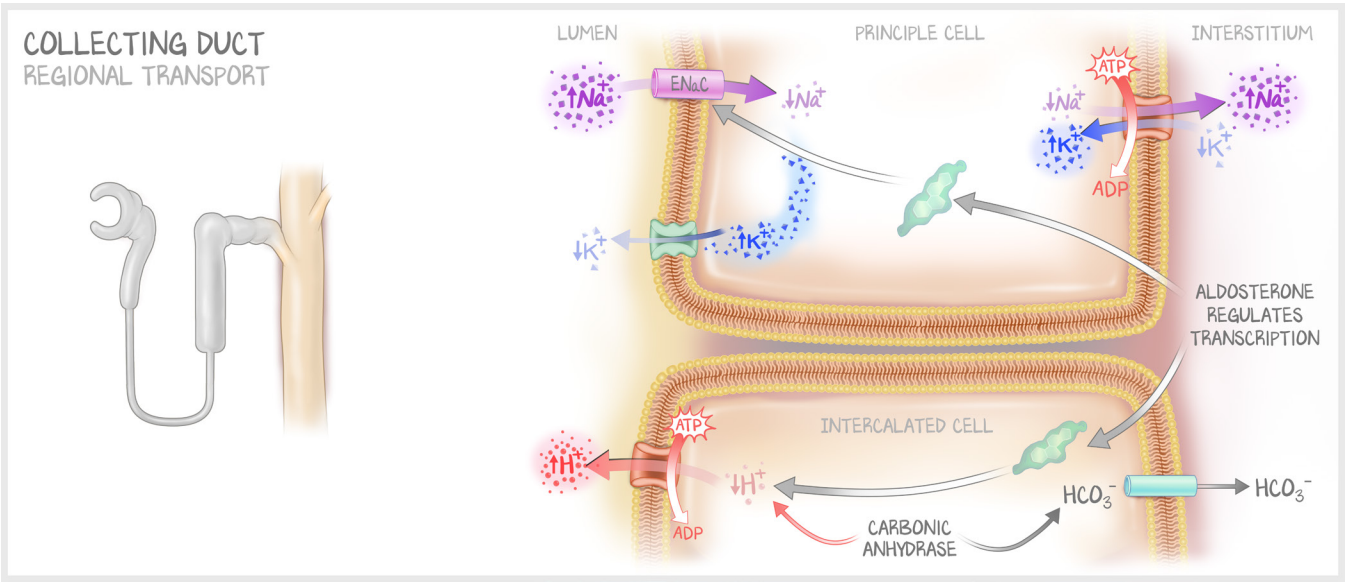


Figure 4.6: CD Regional Transport
In the principal cells, aldosterone induces the expression of ENaC, which passively reabsorbs sodium. When sodium is reabsorbed, potassium is wasted. In the principal cells, antidiuretic hormone places aquaporin channels, which passively reabsorb water. The collecting duct is permeable to both water and sodium, but each only when directed to do so by endocrine signals. Separately, the intercalated cells secrete hydrogen ion under the influence of aldosterone.

Aquaporin channels allow the collecting duct to dilute or concentrate the urine. Having just been in the TAL and DCT, the urine is now dilute. Inserting aquaporin channels would allow more water to be reabsorbed, concentrating the urine. Failure of those aquaporins to reabsorb water would result in water remaining in the lumen, in the urine, diluting the urine. This is mentioned here, as it is a major feature of principal cells, but is discussed in detail in Kidney #5: *Water*.

Carbonic anhydrase 2 is present in the cytoplasm of intercalated cells. When aldosterone is secreted, it activates both principal-cell aldosterone receptors (ENaC insertion) and intercalated-cell aldosterone receptors. Binding of aldosterone to its receptors on intercalated cells results in increased activity of carbonic anhydrase. CO_2 and H_2O are the substrate for carbonic anhydrase. H^+ and HCO_3^- are the products. The H^+ ion is pumped into the lumen, **acidifying the urine**. HCO_3^- is released into the plasma, **alkalinizing the blood**. Unlike in the PCT, that H^+ isn't used for anything. It is simply eliminated.

Therefore, the net effect of the collecting duct (as instructed by aldosterone) is to **reabsorb sodium**, **secrete potassium**, and cause a **metabolic alkalosis** while acidifying the urine. Whereas the other segments simply did what they do, the collecting duct only does what it is able to do when instructed by the RAAS (and ADH).

Pharmacology

The medications we are about to describe have been discussed in Cardiology. Most of them are used to treat hypertension. We are going to review them again in the context of regional transport, explaining in detail their side effects and electrolyte consequences. You should recognize most of these medications. We are including extra detail in these notes that is not included in the video in case you are doing Nephrology before Cardiology.

The earlier in the nephron a medication works, the more potent its ion effects. The collecting duct will always have ENaC channels and K leak channels in it. The collecting duct cannot accommodate excess sodium delivered to it as a result of earlier channels being blocked. It will try. The increased sodium concentration in the lumen caused by blocking sodium reabsorption proximal to the collecting duct will provide a stronger driving force to reabsorb sodium at the collecting duct. More sodium in, more positive charge in, more potassium leaks out. Proximal sodium reabsorption inhibition results in potassium wasting. Sodium reabsorption is the driver for water reabsorption. Natriuresis (sodium lost in urine) and diuresis (water lost in urine) go hand in hand. Drugs that affect regional transport are therefore termed diuretics.

Proximal Convoluted Tubule: Carbonic Anhydrase

Two types of medications work in the proximal convoluted tubule. **Osmotic diuretics** such as **mannitol** (and pathologically, glucose) induce diuresis by having the water follow the osmotic load. In doing so, urine volume increases, and the patient gets volume-depleted. Mannitol is used to control increased cerebral pressure and glaucoma. **Carbonic anhydrase inhibitors** such as **acetazolamide** or **dorzolamide** block hydrogen formation in the PCT; in doing so, H_2CO_3 cannot be regenerated. More specifically, H^+ isn't created, and so reduces the substrate for the H-Na antiporter, leaving Na in the tubules. Because the PCT is responsible for reabsorbing 70% of the sodium in the tubules, blocking this effect has the **most potent natriuretic effect**. It is used as prophylaxis for altitude sickness, glaucoma, and pseudotumor cerebri. This is an assortment of intracerebral pressure changes.

OSMOTIC DIURETICS			
EXAMPLES	**SIDE EFFECTS**	**CONTRA**	**INDICATIONS**
Mannitol	↓ Volume	Limited use	↑ICP

Table 4.1

CARBONIC ANHYDRASE INHIBITORS			
EXAMPLES	**SIDE EFFECTS**	**CONTRA**	**INDICATIONS**
Acetazolamide	↓ Volume	Limited use	Glaucoma
Dorzolamide			Pseudotumor

Table 4.2

2. Loop Diuretics

Loop diuretics are used to treat **volume overload**, as in CHF (peripheral edema, pulmonary edema), or to maintain urinary output in failing kidneys. They work on the **thick ascending limb** of the loop of Henle. They block the **Na-K-2Cl** channels in the apical membrane. With the DCT and CD still to go, this is considered "early" in the nephron, and so, being early, has a **potent natriuretic effect**, and therefore a powerful diuretic effect. Blocking sodium reabsorption early in the nephron leads to an overwhelming of downstream mechanisms, resulting in the loss of sodium in the urine. But because more sodium is delivered to the collecting duct, the concentration gradient is stronger, and more sodium is absorbed. With ENaC channels absorbing more Na, more K is wasted in the urine. Saying it again: even though **diuresis happens because of Na wasting into the urine, the collecting duct also increases sodium reabsorption at the cost of K elimination**. Loop diuretics' biggest risk is **hypokalemia** and hypovolemia (overdoing the diuresis), leading to **prerenal azotemia**.

The **paracellular reabsorption of magnesium** is driven by the Na-K-2Cl transporter. Loop diuretics therefore also can result in **hypomagnesemia**. Finally, giving an intravenous loop diuretic too quickly (physically pushing the IV medication too fast) can lead to **ototoxicity**.

LOOPS			
EXAMPLES	**SIDE EFFECTS**	**CONTRA**	**INDICATIONS**
Furosemide	↓K	AKI	Volume overload
Torsemide	Volume depletion	CKD = OK	CHF, CKD
Bumetanide			Ascites
Ethacrynic acid	↓ Mg ↓ Ca		

Table 4.3

3. Thiazide Diuretics

Thiazide diuretics are used in **hypertension** and also synergistically with loops to potentiate diuresis. Because they function on the **distal convoluted tubule**, later in the nephron than the thick ascending limb, their diuretic potency is not as strong as loop diuretics'. But their mechanism is to block the Na-Cl cotransporter, blocking sodium reabsorption, delivering an increased sodium load to the collecting duct. Therefore, like loops, thiazides can cause **hypokalemia** and can provoke **prerenal azotemia**. Blocking the apical Na-Cl cotransporter puts less Na in the cell. The 3-Na-in, 1-Ca-out antiporter on the basolateral membrane works better with less sodium in the cell. That is, the concentration gradient to remove calcium at the basolateral membrane will be more favorable if sodium is not reabsorbed from the tubules. This **increased absorption of calcium** leads to a **decreased calcium level in the urine**. Since calcium oxalate stones form when there is too much calcium in the urine, and thiazides reduce urinary calcium, they have the added benefit of treating these types of stones. Thiazides have also been known to cause pancreatitis (HCTZ). Thiazide diuretics lose their efficacy above a creatinine of 1.5—which means **don't use thiazides in CKD**.

THIAZIDES			
EXAMPLES	**SIDE EFFECTS**	**CONTRA**	**INDICATIONS**
HCTZ	↓ K	CKD, AKI	HTN
Chlorthalidone	↑bG, ↑Ca	Hypo K	Kidney stones
Indapamide	Pancreatitis ↑ Triglyceride ↓ Urine Ca, ↓ Stones	DM	Nephro DI

Table 4.4

4. Angiotensin-Converting Enzyme Inhibitors

ACE inhibitors are the standard treatment for hypertension and are indicated in almost all comorbid conditions—diabetes, nephropathy, proteinuria, heart failure, coronary artery disease, etc. They are also the most tested medications. ACE inhibitors **prevent peripheral activity of angiotensin-2**, leading to reduced systemic vascular resistance, and therefore combat hypertension. But they also **prevent activation of aldosterone**. This reduces the number of ENaC channels in the collecting duct, reducing reabsorption of Na and wasting less potassium. Thus **ACE-i's can cause hyperkalemia**. They also reduce the effect of ANG-2 on the afferent and efferent arterioles, leading to vasodilation. Vasodilation of both arterioles **increases renal blood flow**, but at the cost of reducing the hydrostatic forces at the glomerulus, thereby **reducing GFR**. The afferent arteriole is less sensitive to angiotensin-2 (because of the myogenic response) than the efferent arteriole. Therefore, inhibition of angiotensin-2, which inhibition has a vasodilatory effect, vasodilates the efferent more than the afferent. Renal blood flow increases, but filtration forces are reduced. A rise of the creatinine by 10% is expected and considered normal. The kidneys are better perfused, but the lab test we use to assess kidney function (the creatinine) makes it look like they are doing worse.

Failing kidneys retain the ability to make renin. The only thing a dying kidney can do is make the hormone that ultimately limits the perfusion to the kidney. The kidney is not smart. ACE inhibitors are therefore indicated **in all CKD**, except for CKD stage 4, where that expected loss of 10% of GFR may result in anuria, progressing the patient to end-stage renal disease. Once CKD stage 5, once they are on dialysis, ACE inhibitors are absolutely indicated.

Specific to ACE inhibitors is the **chronic dry cough** that can accompany them. Switch to an ARB if a patient is on an ACE-i and develops a chronic cough. Also unique to ACE-i's, because of their impact on the bradykinin pathway, is that they can induce **angioedema**, a life-threatening condition if it involves the mouth or airway.

ACE INHIBITORS			
EXAMPLES	**SIDE EFFECTS**	**CONTRA**	**INDICATIONS**
Lisinopril	↑ K	Renal artery stenosis	CHF, CKD
Captopril	Dry cough		Diabetes, ESRD
Quinapril	Angioedema	Angioedema	Proteinuria
	↑ Cr	CKD stage 4	

Table 4.5

5. Angiotensin-Receptor Blockers

ARBs are effectively **the same as ACE-i's**, except they do **NOT** cause angioedema or cough.

ANGIOTENSIN-RECEPTOR ANTAGONISTS			
EXAMPLES	**SIDE EFFECTS**	**CONTRA**	**INDICATIONS**
Valsartan	↑K	Renal artery stenosis	CHF, CKD
Candesartan	↑Cr		Diabetes, ESRD
		CKD stage 4	Proteinuria

Table 4.6

6. Aldosterone Antagonists

These medications work directly on the **collecting duct** by inhibiting the insertion of ENaC channels. Like ACE-i's and ARBs, it will cause **hyperkalemia**. Its main mechanism of action is to inhibit reabsorption of sodium (and therefore water), reducing preload, and thereby reducing blood pressure. Because it is late in the nephron, its diuretic potency is poor, although its antihypertensive effect is strong. **Spironolactone** can cause **gynecomastia**. If this occurs, switch to **eplerenone**.

ALDOSTERONE ANTAGONISTS			
EXAMPLES	**SIDE EFFECTS**	**CONTRA**	**INDICATIONS**
Spironolactone	↑K		CHF class III
Eplerenone			Ascites

Table 4.7
Spironolactone = Gynecomastia, Eplerenone = None

7. K-Sparing Diuretics

Triamterene and **amiloride** are collecting-duct medications that, when used in combination with thiazides, can help with blood pressure and keep the potassium at a normal level. They aren't useful diuretics or antihypertensives, and so they are never used on their own. They help reduce the potassium side effects of thiazides. Since the advent of better, more potent anti-collecting-duct medications (ACE-i's, ARBs, aldosterone antagonists), these medications have been effectively eliminated from treating patients.

K-SPARING			
EXAMPLES	**SIDE EFFECTS**	**CONTRA**	**INDICATIONS**
Triamterene	↑K if used alone	CKD-IV	Adjunct to K-wasting diuretics
Amiloride			

Table 4.8